AN INTEGRATED LC-ESI CHIP WITH ELECTROCHEMICAL-BASED GRADIENT GENERATION

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ABSTRACT

This work reports the first on-chip generation of gradient elution using electrochemical pumping, for application in Liquid Chromatography - Electrospray Ionization (LC-ESI). The system consists of Parylene, SU-8 and PDMS parts to create two 3 μL on-chip solvent reservoirs, two electrochemical pumps and an ESI nozzle. Pumping is controlled galvanostatically (i.e., through electrolysis current). At low back pressures, pumping rate can reach 200 nL/min using just 40 μA (power consumption <100 μW). At 80 psi, 200 μA (power consumption <1 mW) is sufficient to deliver fluid at 75 nL/min. Higher electrical currents have been used to increase the flow rate or to achieve pumping at higher back pressures. For example, pumping at 200 psi back pressure has been demonstrated using a current of 800 μA. Electrically controlled on-chip gradient generation is achieved without any external fluidic connections to the chip. Continuous generation of gradients for 30 minutes at a total flow rate of ~100 nL/min is also realized and confirmed with mass spectroscopy (MS) measurements. The developed on-chip gradient generation system meets the general requirements of LC applications.

1. INTRODUCTION

Liquid Chromatography has proven to be one of the most important analytical tools in proteomics. Miniaturization of LC is essential to meet the demand of fast growing proteomic research and development [1]. Although there are many attempts to create an on-chip LC system, a complete on-chip LC system has yet to be realized. Among various difficulties, high-pressure on-chip fluidic pumping is the bottleneck preventing total integration [2].

In most LC separations, especially reverse-phase LC, gradient elution is usually adopted [3]. Figure 1 shows the principle components of a LC-ESI system and a typical solvent gradient profile. In the case of reverse-phase LC, the gradient begins at a high aqueous concentration and the organic concentration is slowly increased over time. Conventional LC pumps generally operate at pressures >1000 psi. For on-chip LC systems though, a flow rate of 50 nL/min at 200 psi should be adequate, due to a shorter separation column. Mechanical pumps, including electrostatic, thermopneumatic, electromagnetic, piezoelectric actuators, etc., often involve moving parts and are very difficult to integrate on a chip. Moreover, most mechanical pumps cannot generate reasonable pumping rates at high back pressures without consuming significant power. Because of this, most previous approaches to on-chip LC pumping exploit electroosmotic force, which unfortunately often requires high voltages (>1000 Volts) and a packed porous material. Electrochemical actuation in microfluidics [4-6] is promising, but little, if any, has been done to apply it to an on-chip high-pressure pumping system for LC applications.

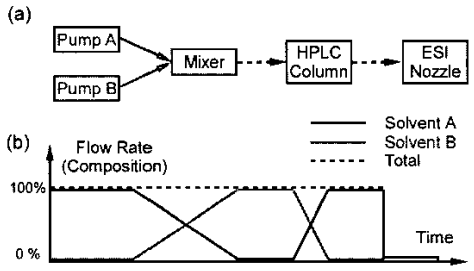

Figure 1. *(a) Principle components of a LC-ESI system. (b) A typical solvent gradient profile. Normally, solvent A is mostly aqueous and B contains high organic concentration.*

To fabricate the various microfluidic components, such as an ESI nozzle, a mixer, and solvent chambers, onto a single chip, several polymer micromachining technologies are utilized. Methods are developed to seamlessly integrate these different polymer materials and technologies together.

In this work, we achieve on-chip high-pressure pumping by using pneumatic pressure generated from the electrolysis of water. Current controlled high-pressure pumping is demonstrated. Without any external fluidic connections to the chip, electrically controlled on-chip gradient generation is successfully produced and verified using MS measurements.

2. DESIGN AND FABRICATION

A schematic of the system is shown in Figure 2. There are three main components. First, there is a molded PDMS piece that serves as the solvent chamber and gasket. The recessed PDMS areas define the chamber volume. The second piece is a glass cover, which is used to clamp the PDMS piece in place. Then, we have a silicon substrate on which an ESI nozzle, a mixer, and electrolysis electrodes are integrated. The silicon substrate is further planarized using thick SU-8 layer to create a flat surface for the PDMS piece to sit on.

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To use the chip, first the PDMS cover is put on the SU-8 planarized chip substrate. The adhesion between the PDMS and the SU-8 is adequate for filling the chambers with a syringe. Once the solvent chambers are filled, the PDMS piece is clamped down using the glass cover to ensure the solvent chambers are sealed.

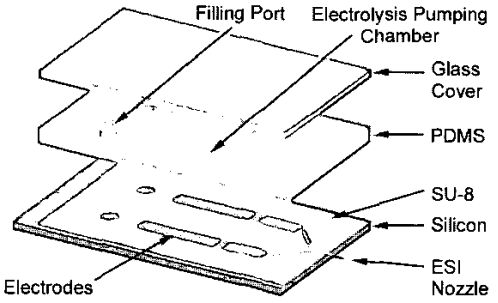

Figure 2. *The schematic of the integrated system having various components made from several polymers.*

The fabrication process of the chip begins with a 4-inch silicon wafer with 1.5 μm thermally grown oxide. Electrolysis electrodes are made of thermally evaporated Gold/Titanium (4000 Å /100 Å). After patterning the electrodes, the front side oxide is patterned using buffered HF. In order to promote adhesion between the silicon substrate and the succeeding parylene layer, short XeF_2 dry etching is performed to roughen the exposed silicon surface. Two layers of Parylene (4.5 μm each) with a sacrificial photoresist (5.5 μm) in between form an ESI nozzle, a passive mixer, as well as the channels, which connect the nozzle/mixer to the solvent chambers. A thick SU-8 layer (100 μm) planarizes the overall chip surface to support the PDMS cover. After the sacrificial photoresist is dissolved, the 1 mm long overhanging nozzle is undercut and made freestanding using XeF_2 dry etching. The PDMS cover is cast using a 200 μm high SU-8 mold on another wafer. The actual device and fabrication process are shown in Figure 3 and 4.

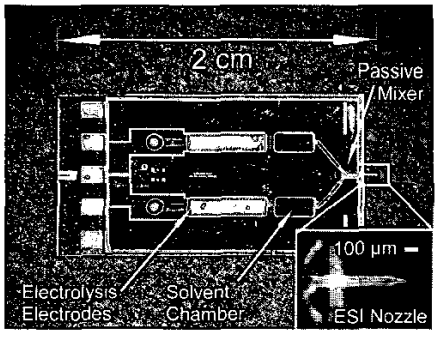

Figure 3. *Pictures of the fabricated microfluidic chip. The insert is a micrograph of the ESI nozzle.*

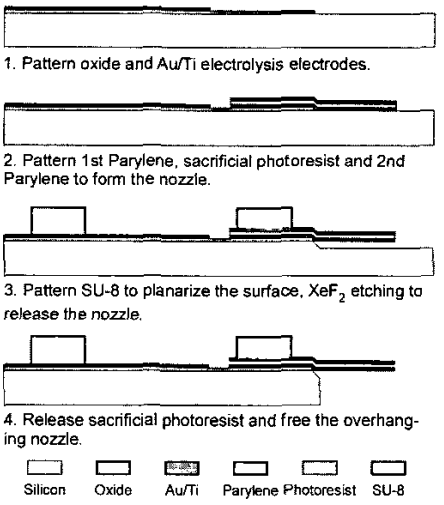

Figure 4. *Fabrication process of the integrated microfluidic chip.*

Several process steps require extra attention. First, since cross-linked SU-8 films are highly stressed, serious cracking in the underlying parylene structures can occur if these structures coincide with the edge of the SU-8 layer. To solve this, baking steps for the SU-8 are carried out under much lower temperatures (70 °C) and slower ramping speeds (2 °C/min) than the manufacturer's guidelines suggest (95 °C, >10 °C/min). Secondly, acetone, which is generally used to dissolve away sacrificial photoresist, causes large volume change of the SU-8 layer. This can result in cracks or delamination of the SU-8 from the substrate. So instead of acetone, SU-8 developer (propylene glycol monoether acetate, PGMEA) is used. One side effect of this substitution is a longer photoresist dissolution time. This may be due to weaker solvent strength and/or slower diffusion of the dissolved photoresist. Thirdly, since this process includes two Parylene structural layers and one SU-8 layer, to ensure good adhesion between these layers, careful cleaning is needed. Cleaning is done using oxygen plasma etching to roughen the surface, followed by a 5 % HF dip.

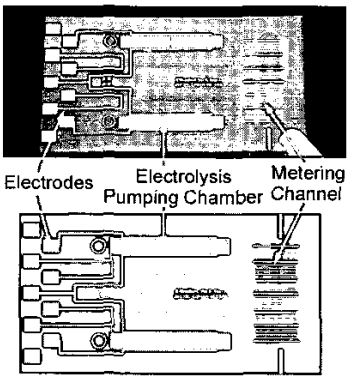

Figure 5. *Picture of the fabricated testing chip with metering channel for fluidic measurement.*

To facilitate characterization of electrochemical pumping, a testing chip that replaces the ESI nozzle with a meandering metering channel was also fabricated. In this testing chip E-beam evaporated Platinum/Titanium (2000 Å /300 Å) is used for the electrolysis electrodes instead of Gold/Titanium. Picture of the fabricated testing chip is shown in Figure 5.

3. TESTING AND RESULTS

Measurements of the pumping rate as a function of applied current at low pressure (<1 psi) are performed using the testing chip and the results are shown in Figure 6. The testing chip has a metering channel with gradations to monitor the meniscus movement from which the pumping rate is calculated. A common LC solvent composition (95/5/0.1% water/acetonitrile/formic acid) is used as the electrolyte. Pumping rates ranging from 40 to 200 nL/min are observed for currents between 15 and 40 μA. At these currents, power consumption is <100 μW. In an ideal electrochemical system, the volume of gas generation is proportional to the total charge injected. But due to recombination, joule heating, leakage through gas permeable PDMS, etc., the achieved pumping rate is much less than that of the ideal case. Here, the pumping efficiency is calculated by dividing the volume of the liquid displaced, by the volume of gas that should have been generated (assuming the electrolysis of pure water and based on the charge injected). The efficiency, given by Table 1, ranges from 0.22 to 0.40 and is also found to increase with increasing current value.

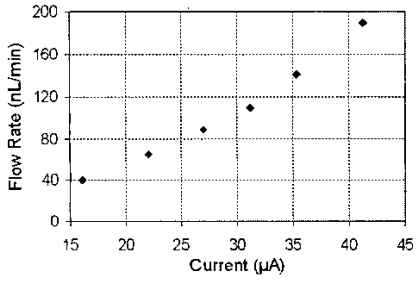

Figure 6. *Pumping rate of the electrochemical pump for different currents at low pressure.*

Table 1. *Pumping raet and efficiency at different currents. Ideal rate is calculated assuming electrolysis of pure water and is based on the charge injected*

Current (μA)	Flow Rate (nL/min)	Ideal (nL/min)	Pumping Efficiency
41.2	190	474	0.40
35.4	141	407	0.35
31.2	110	359	0.31
27	89	310	0.29
22.1	65	254	0.26
16.1	40	185	0.22

High-pressure pumping is characterized using a modified testing setup in which the testing chip pumps liquid into a piece of thin tubing attached to a regulated pressure source. By doing this, the back pressure can be varied and resulting changes in pumping rate can be measured. The pumping rate as a function of both current and applied back pressure is shown in Figure 7. As expected, higher currents are needed to generate the same pumping rate at higher back pressures. The current can also be increased to achieve higher pumping rates or to pump at higher pressures. Currents as high as 800 μA have been used to demonstrate a pumping rate of 20 nL/min at 200 psi back pressure (power consumption <4 mW) and even higher currents are not out of the question. Currently the highest pressure we can test at is 200 psi and that is limited by the robustness of our experimental setup.

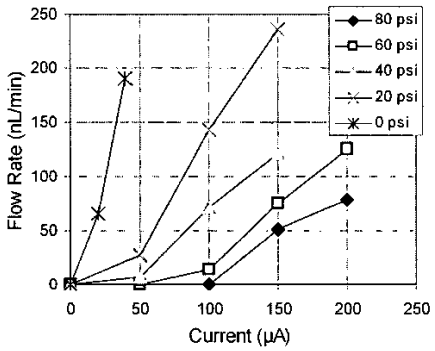

Figure 7. *Pumping rate of the electrochemical pump for different currents at high pressure.*

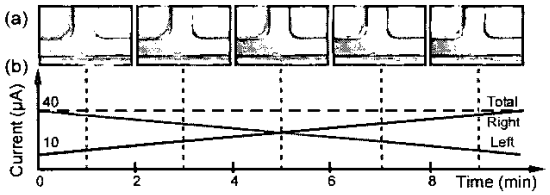

Figure 8. *(a) Visualization of gradient elution. Solvent being pumping from left side is dyed green. (b) The control current profile. The video snapshots are taken at the moments indicated by the dash lines.*

Gradient generation can also be visualized using green dye as shown in Figure 8. For these visualizations the testing chip is used. Liquid pumped out from two pumping chambers is merged at a T-junction, the left chamber having green dye. Due to the laminar flow nature, the composition of the merged flow can be deduced from the width of the two streams. The gradient formation corresponds well to the two control currents.

Finally, gradient generation has been demonstrated using solvent mixtures commonly used in LC applications. These include solvents of high organic concentration (acetonitrile or methanol). The pumped solvents are coupled into a MS using the integrated ESI nozzle. The solvents are marked with unique chemical species, which are detected by the MS, to determine the relative pumping rates. The MS output offers confirmation of successful gradient generation. Figure 9 shows multiple gradient generations with an average total flow rate of ~100 nL/min. One reservoir is filled with a 90/10/0.1% (Water/Acetonitrile/Formic Acid) solution marked with TBAI (10 pmol/μL, m/z 242). The other is a 95/5/0.1% (Water/Methanol/Formic Acid) solution marked with Angiotensin (25 pmol/μL, m/z 433). Continuous gradient generation for 30 minutes is achieved. The generated gradient corresponds well to the control current profiles. Similar results were also obtained with high organic solvents (up to 50% acetonitrile). The demonstrated performance compares favorably with existing capillary systems and meets the requirements of most LC-ESI applications.

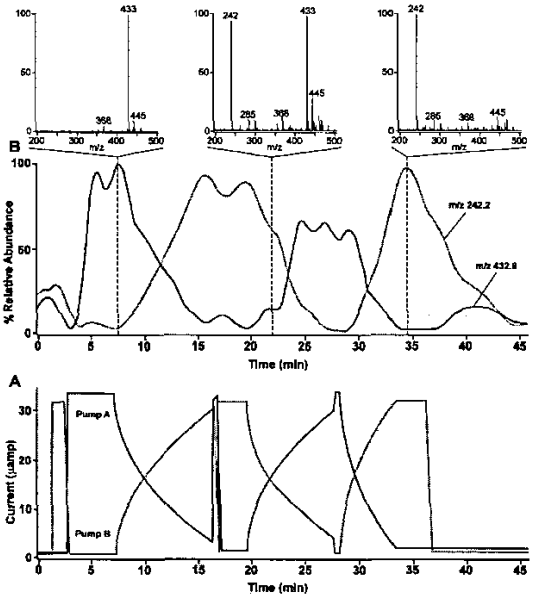

***Figure 9**. Gradient generation for LC-ESI-MS. (a) The control current profile used. (b) Chromatogram shows the intensity of the chemical markers used in the solvents as seen by the MS. Mass spectra taken at the moments indicated by the dash lines during gradient generation are also shown.*

4. CONCLUSIONS AND DISCUSSION

On-chip, high-pressure, electrochemical pumping has been successfully demonstrated. Pumping control using electrolysis current proves to be reliable and effective. The application of these electrochemical pumps for on-chip gradient generation in micro scale LC is realized using an integrated microfluidic chip. Various polymer micromachining technologies are adopted to integrate all the essential components, such as an ESI nozzle, mixer, channels and solvent chambers, on a single chip.

Future work will focus on integrating a separation column onto the chip and achieving separation using the gradient generated from the presented electrochemical pumps. Sample injection will also be implemented using a similar scheme. Some materials used in the current system are not chemically resistant enough, thus they are not suitable for demanding LC applications. Alternative materials and micromachining technologies need to be developed in order to make an on-chip LC-ESI system that can perform the analytical work done by today's macro scale LC systems.

ACKNOWLEDGEMENT

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